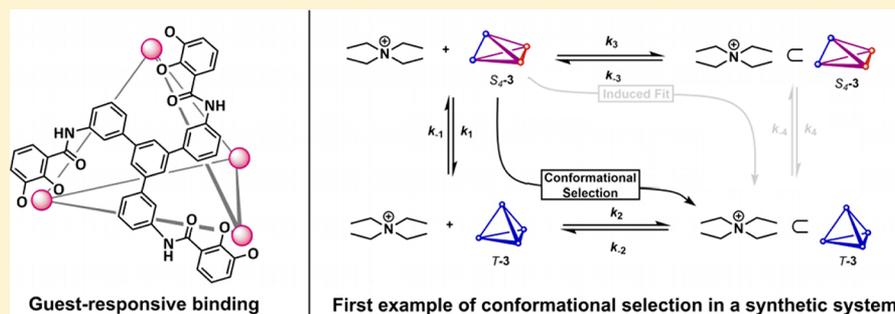


Conformational Selection as the Mechanism of Guest Binding in a Flexible Supramolecular Host

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 Supporting Information



ABSTRACT: This study offers a detailed mechanistic investigation of host–guest encapsulation behavior in a new enzyme–mimetic metal–ligand host and provides the first observation of a conformational selection mechanism (as opposed to induced fit) in a supramolecular system. The Ga_4L_4 host described features a C_3 -symmetric ligand motif with *meta*-substituted phenyl spacers, which enables the host to initially self-assemble into an S_4 -symmetric structure and then subsequently isomerize to a T -symmetric tetrahedron for better accommodation of a sufficiently large guest. Selective inversion recovery ^1H NMR studies provide structural insights into the self-exchange behaviors of the host and the guest individually in this dynamic system. Kinetic analysis of the encapsulation–isomerization event revealed that increasing the concentration of guest inhibits the rate of host–guest relaxation, a key distinguishing feature of conformational selection. A comprehensive study of this simple enzyme mimic provides insight into analogous behavior in biophysics and enzymology and aims to inform the design of efficient self-assembled microenvironment catalysts.

INTRODUCTION

The understanding of protein–ligand molecular recognition is of paramount importance to the study of enzymatic catalysis and allosteric regulation of cell signaling as well as to the design of more efficient drugs.¹ The high levels of specificity observed in enzyme catalysis and protein–ligand binding have been classically accepted to proceed predominantly by the “induced fit” hypothesis, introduced in 1958 (also referred to as the Koshland–Nemethy–Filmer model), rather than a concerted binding model.^{2,3} The induced fit model stipulates that a protein encounters its ligand in an inactive state, and an optimum fit is achieved only after structural rearrangement is induced by the interaction with the ligand (Figure 1). Seven years later, an alternative mechanism for ligand recognition was proposed, referred to as “conformational selection” or the Monod–Wyman–Changeux model.⁴ This mechanistic hypothesis postulates that the enzyme exists in solution as a dynamic set of Boltzmann-populated conformations, and the ligand selectively interacts with the active conformation of the protein to form the ligated complex and subsequently shifts the equilibrium distribution of protein conformations.

Until recently, conformational selection was considered by some to be an uncommon mechanism, with few definitive examples reported in the literature.⁵ However, single molecule fluorescence^{6,7} and NMR relaxation^{8,9} experiments in the past decade have resulted in the accumulation of significant evidence in support of conformational selection as a competing mechanism for protein–ligand interactions.^{1,10} While indirect evidence for conformational selection can be garnered by techniques including crystallographic analysis, selective mutation of receptors, and *in silico* molecular dynamics, the most compelling evidence to distinguish the induced fit and conformational selection mechanisms arises from the kinetic profile of the binding event. Specifically, the rate of approach to the binding equilibrium shows saturation kinetics in ligand concentration for induced fit and inverse dependence on ligand concentration for the conformational selection mechanism (Figure 2). Deciphering the mechanism of protein–ligand recognition in the context of these two general mechanisms has

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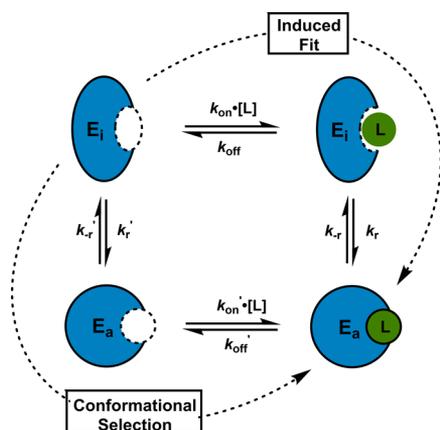


Figure 1. Induced fit and conformational selection mechanisms of substrate binding.

since become a very active topic of study in biophysics and enzymology.^{1,4}

The mechanisms for induced fit and conformational selection each consist of two consecutive reversible reactions, where guest binding precedes isomerization in induced fit binding and isomerization precedes guest binding in conformational selection. A complete description of their respective rate laws is beyond simple analysis and can be found in the literature.¹¹ However, under the “rapid equilibrium approximation”, a kinetic scenario that often prevails, expressions for the binding rate constant (k_{obs}) can be derived (Figure 2, eqs 1–3). Specifically, the rapid equilibrium approximation makes the simplifying assumption that the ligand-binding step is fast and reversible on the time scale of the conformational change, allowing the consecutive equilibria to be treated independently (see the Supporting Information).^{5,12,13}

For an induced fit mechanism, the rate law for relaxation of inactive enzyme (E_i) to the active enzyme (E_a) bound to substrate (L) can be expressed by applying a pseudoequilibrium approximation to the guest binding step. This enables an expression for the concentration of intermediate E_iL in terms of $[E_{i,\text{total}}]$ and $[L]$. The concentration of $[E_iL]$ can then be used to express the overall rate constant as the sum of the forward and the backward rate constants for conformational change.¹⁴ The rate law for an induced fit mechanism shows saturation kinetics in ligand concentration in the forward direction, while the reverse reaction is independent of ligand. This results in overall saturation kinetics with respect to ligand concentration.

In contrast, a similar analysis applied to the mechanism for conformational selection reveals that the forward reaction is

independent of ligand concentration, while the reverse reaction is inhibited by the ligand. The resulting rate constant describing the approach to equilibrium under the conformational selection mechanism shows overall partial inverse order in ligand concentration. While counterintuitive, this prediction has been experimentally validated in the molecular biology literature.^{12,13,15,16}

The symbiotic relationship between molecular biology and supramolecular chemistry suggests that similar kinetics scenarios might arise in dynamic synthetic systems. Simple biomimetic supramolecular assemblies have served as model systems to provide insight into the underlying stereoelectronic interactions that drive molecular recognition and enzymatic reactivity in biological systems, and in turn, lessons from biology have contributed to the design of synthetic receptors and catalysts. Self-assembled nanovessels, including hydrogen bond and metal–ligand based assemblies, have been particularly fruitful as models for the study of molecular recognition and microenvironment catalysis.^{17–21}

The Raymond, Toste, and Bergman collaboration has extensively studied the host–guest chemistry and catalytic behavior of $K_{12}Ga_4L_6$ tetrahedral assembly **1**, which is composed of six biscatecholate ligands bridging four homo-chiral ($\Delta\Delta\Delta\Delta$ or $\Lambda\Lambda\Lambda\Lambda$) pseudo-octahedral gallium(III) atoms (Figure 3A).²² Assembly **1** features a range of enzyme-

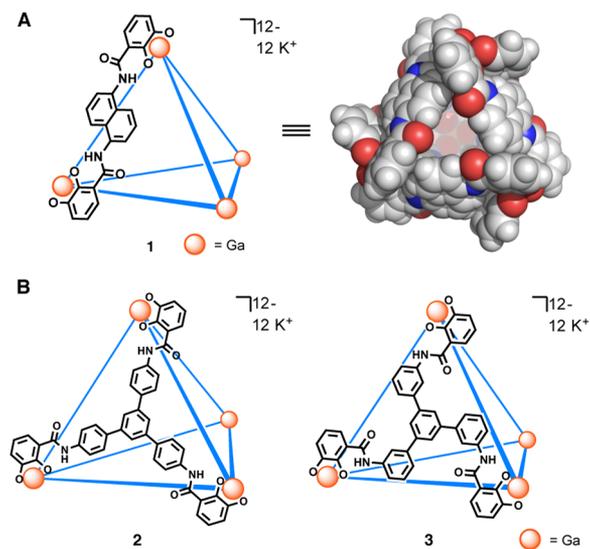


Figure 3. (A) Tetrahedral M_4L_6 supramolecular host **1**, (B) previously reported M_4L_4 host **2**, and isomeric host **3**.

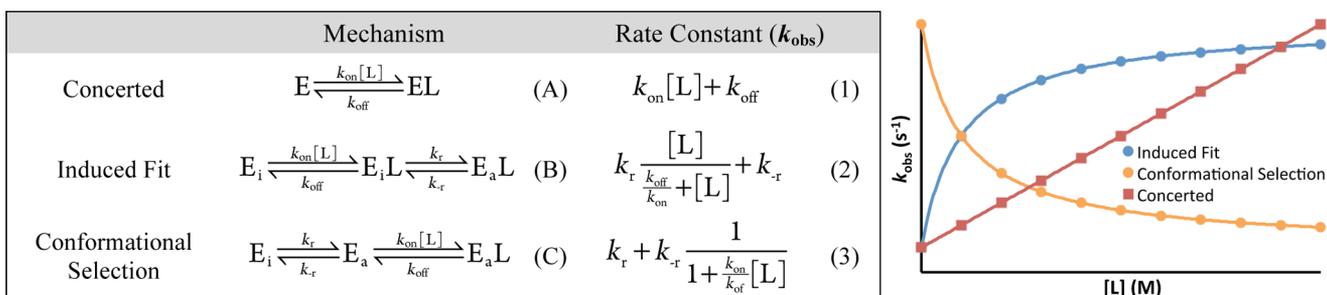


Figure 2. Observed rate constants (k_{obs}) for an approach to substrate binding equilibrium under concerted, induced fit, and conformational selection mechanisms (left) and qualitative comparison of observed rate constants as a function of ligand concentration for induced fit (blue dots), conformational selection (orange dots), and concerted binding (red squares) mechanisms (right).

mimetic behaviors including hydrophobic encapsulation of neutral and anionic guests,^{23,24} Michaelis–Menten-type mechanisms for a range of catalytic applications with rate accelerations ($k_{\text{cat}}/k_{\text{uncat}}$) of up to 1.9×10^7 fold,^{25–27} unusual product selectivity reminiscent of enzymatic catalysis,^{28–30} and even protein-like amide H–D exchange behavior.³¹ While assembly **1** has proven a fruitful supramolecular enzyme mimic, it is inherently limited with respect to the steric and electronic properties of the catalyst active site. While structural analogues of **1** have been reported,^{32,33} the rapid diversification of novel M_4L_6 assemblies is significantly impaired by the fact that tetrahedra of M_4L_6 stoichiometry are entropically disfavored relative to the M_2L_3 helicate. Only the carefully tuned geometry of the internal spacer in the bis-bidentate ligand of **1** prevents fragmentation to the helicate, and subtle variations from this structure often leads to the incomplete self-assembly of the tetrahedron or selective formation of the M_2L_3 helicate.³⁴

In contrast, M_4L_4 tetrahedra assembled from C_3 -symmetric tris-bidentate ligands are less likely to suffer from this entropic complication, given that the ligands are sufficiently rigid. The Raymond group has previously reported the synthesis of M_4L_4 assembly **2** (Figure 3B); however, the scope of host–guest chemistry observed with **2** was prohibitively limited in comparison with assembly **1**.³⁵ We hypothesized that the disparity in encapsulation between these hosts may be attributed to a difference in the innate flexibility or “breathability” of their cavity structures. In much the same way that a highly specific protein–ligand interaction requires that the protein undergo a configurational change to accommodate and conform to the structure of its ligand (vide supra), so too should supramolecular hosts conform to complement their guest molecules for optimal binding.

In assembly **1**, the amide substitution on the central naphthalene linker is offset from the center of the ligand, which allows the walls of the cavity to expand and contract through rotation about the amide–aryl nitrogen–carbon bond. This is evidenced by the crystallographic observation that the encapsulated guest inside **1** can influence the cavity volume within from at least 253 to 435 Å.³⁶ In contrast, the amide–aryl bonds within the ligand of assembly **2** are oriented radially from the origin of C_3 -symmetry, and as a result, any structural accommodation of guest molecules must occur predominantly by enthalpically costly bond distension. We therefore hypothesized that the introduction of rotational flexibility to the M_4L_4 structural manifold might reintroduce the “breathability” required for efficient guest encapsulation while opening the opportunity for host diversification by maintaining the benefits of entropic stability.

In accordance with this hypothesis, tetrahedral assembly **3** was selected as a synthetic target by self-assembly of ligand **4** (Figure 3). Host **3** is isomeric to assembly **2**, differing only in the *meta*-substitution pattern of the trianiline-linker that generates the C_3 -symmetry of ligand **4** (as opposed to *para*-substitution in the ligands of host **2**). This *meta*-substitution pattern introduces 60° of curvature in each arm of the ligand and offsets the metal binding moiety from the center of the ligand. This was expected to grant assembly **3** the necessary flexibility to conform to guest molecules without incurring a significant enthalpic penalty. We discuss here the dynamic structural consequences of this simple isomeric substitution, including the observation of a guest-induced structural reorganization upon encapsulation of an appropriately large guest molecule. Mechanistic investigation of the guest-induced

host reorganization supports the conclusion that a conformational selection mechanism of molecular recognition is operative, the first such observation in the context of synthetic supramolecular enzyme mimics.

RESULTS AND DISCUSSION

With ligand **4** in hand, the synthesis of host **3** was attempted under the established self-assembly conditions. Ligand **4** and $\text{Ga}(\text{acac})_3$ in equimolar quantities were suspended in degassed methanol- d_4 followed by the addition of 3 equiv of potassium hydroxide as a methanolic solution. This gave rise to spontaneous self-assembly to form a single species, assigned as host **3**, which could be isolated by precipitation with acetone. However, the ^1H NMR spectrum of the resulting assembly revealed the presence of 24 unique resonances, which is inconsistent with the eight unique chemical environments of a M_4L_4 host of T -symmetry (Figure 4, top). This low-symmetry

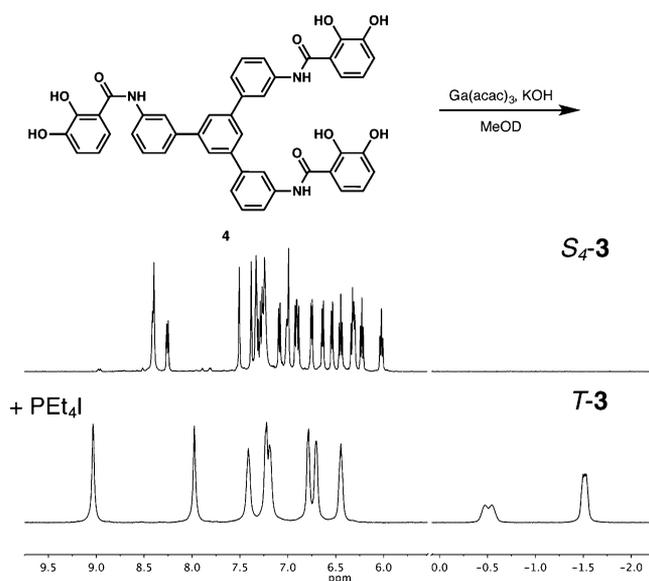


Figure 4. ^1H NMR spectrum of host **3** immediately after assembly (top) and after the addition of approximately 1.5 equiv of tetraethylphosphonium iodide (bottom).

species was persistent, even upon heating in methanol for several days (60 °C). Furthermore, the addition of potential guest cation tetraethylphosphonium resulted in a rapid reduction in the number of proton resonances from 24 to 8, along with the appearance of two new resonances around -0.5 and -1.5 ppm (Figure 4, bottom). These resonances and their integrations were indicative of a molecule of tetraethylphosphonium encapsulated within host **3**, with ^1H NMR resonances shifted upfield due to close contact with the aromatic ring currents of the assembly walls. These observations are consistent with formation of T -symmetric M_4L_4 inclusion complex $\text{PEt}_4^+ \text{C} \text{3}$; however, the structure of the initially formed low symmetry species remained unclear.

Characterization of Assembly 3. Three potential structures might explain the species observed from host **3** in the absence of a guest. First, the increased flexibility of ligand **4** might alleviate the mechanical coupling that enforces homochirality on the $\text{Ga}(\text{III})$ centers in assemblies **1** and **2**; if this is the case, then assembly **3** might adopt the S_4 -symmetric, mixed metal-chirality isomer ($\Lambda\Lambda\Delta\Delta$) in the absence of guest. Second, it is also plausible that a reduction

in overall symmetry of host **3** may arise from a collapse of the flexible ligand walls while maintaining homochirality of the Ga(III) metal centers. This conformational variation would reduce the host to D_2 symmetry and might arise from pinching two pairs of vertices together. Lastly, it is also conceivable that if the ligand **4** is sufficiently flexible, an M_2L_2 structure of D_2 symmetry could initially form under self-assembly conditions. This would require that the addition of a guest initiates a rapid rupture and dimerization of two complexes to form the observed inclusion complex $PEt_4^+ \subset T\text{-3}$.

Diffusion-ordered NMR spectroscopy (DOSY) and electrospray mass spectrometry (ESI-MS) were employed to differentiate the predicted M_4L_4 host **3** from this potential M_2L_2 structure. It was anticipated that a low-symmetry M_4L_4 host would have a similar hydrodynamic radius and therefore rate of diffusion compared to those of the inclusion complex $PEt_4^+ \subset T\text{-3}$. In contrast, an M_2L_2 complex would have a significantly smaller hydrodynamic radius and thus diffuse much more quickly. DOSY NMR revealed a diffusion coefficient of $1.52 \times 10^{-5} \text{ cm}^2/\text{s}$ for low symmetry host **3**, while the complex $PEt_4^+ \subset T\text{-3}$ had a diffusion coefficient of $1.96 \times 10^{-5} \text{ cm}^2/\text{s}$. The proximity of these values was a strong indication that the M_4L_4 stoichiometry is preserved between two forms of **3**. ESI-MS measurements of the low symmetry host **3** corroborated this conclusion, as signals consistent with an M_4L_4 host were observed.³⁷ These data together supported the conclusion that the host **3** adopts a low symmetry structure in the absence of guest.

An S_4 -symmetric $\Lambda\Lambda\Delta\Delta$ conformation of host **3** may form a “collapsed” assembly, reducing the internal cavity volume and, by extension, reducing the entropic penalty incurred by trapping solvent molecules inside. Desymmetrization of the assembly from T to S_4 symmetry would be consistent with the 3-fold increase in unique proton resonances upon isomerization. While the majority of M_4L_4 and M_4L_6 tetrahedra exhibit T symmetry due to ligand-enforced mechanical coupling, examples of M_4L_6 assemblies have been reported to exist as near-statistical mixtures of T - ($\Delta\Delta\Delta\Delta/\Lambda\Lambda\Lambda\Lambda$), C_3 - ($\Lambda\Delta\Delta\Delta/\Delta\Lambda\Lambda\Lambda$), and S_4 -symmetric ($\Lambda\Lambda\Delta\Delta$) structures.^{38–42} To the best of our knowledge, no M_4L_4 or M_4L_6 assembly has been reported in which the S_4 -symmetric $\Lambda\Lambda\Delta\Delta$ isomer is observed exclusively.

Similarly, a D_2 -symmetric conformation of host **3** arising from a pair of pinched vertices and collapsed ligands would also reduce the internal cavity volume. If indeed the D_2 -symmetric structure was an energetic minimum for host **3**, the discrimination of these structures with ^1H NMR would require that their barrier of interconversion be significantly higher than would be expected for a process invoking simple conformational exchange of ligand **4** within host **3**.

Conformational Fluxionality of Host 3. To interrogate the configurational changes that give rise to the reduction of overall symmetry in **3**, the dynamics of host isomerization were studied. Because the C_3 -symmetry of ligand **4** is broken upon self-assembly into host **3**, the unique chemical environments created by this desymmetrization were expected to be related by conformational exchange of each unique ligand resonance in the cases of both S_4 - and D_2 -symmetric proposed structures of host **3**. If the low symmetry of host **3** is a consequence of mixed Ga(III) chirality ($\Delta\Delta\Lambda\Lambda$), then the exchange process that interconverts the desymmetrized protons was anticipated to proceed via stepwise Bailar twists by analogy to studies in the literature on the mechanisms of Ga(III) triscatecholate and

helicate isomerizations.^{43,44} The first Bailar twist from S_4 -symmetric **3** would generate a C_3 -symmetric intermediate, and a second Bailar twist would then provide either the T -symmetric **3** or a second isomeric S_4 -symmetric host **3** (Figure 5).

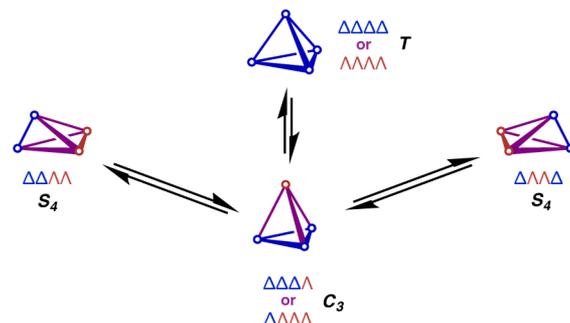


Figure 5. Stepwise Bailar twist mechanism for $\Delta\Delta\Lambda\Lambda$ -**3** degenerate isomerization.

It should be noted that the plausibility of an empty T -symmetric host **3** as an intermediate in the self-exchange of S_4 -**3** depends on the relative energetic barriers for the S_4 - C_3 Bailar twist and the C_3 - T Bailar twist. Because these relative barriers could not be probed, analysis of the kinetics parameters of self-exchange do not necessarily directly inform the barrier of S_4 - to T -symmetric host isomerization involved in guest binding. However, as these processes are mechanistically related, these self-exchange parameters are taken to shed a qualitative light on the kinetic parameters of this process.

The degenerate isomerization of **3** was initially established by the observation of ^1H NMR peak coalescence at elevated temperatures. In D_2O , the 24 observed resonances of **3** coalesced into eight broad peaks at approximately 90°C , implying the observation of the time-averaged T -symmetric conformation from rapid S_4 isomerization (see the [Supporting Information](#)). However, line-broadening effects and complications with the temperature dependence of isotropic chemical shifts prevented an accurate determination of kinetic activation parameters from this method.

To avoid these issues, the technique of selective inversion recovery (SIR) ^1H NMR was employed to measure the isomerization kinetics of the low symmetry host **3** (Figure 6). SIR experiments provide an excellent method of measuring rates of slow-exchange (rates of 0.01 s^{-1} to 100 s^{-1}) in resolved systems at equilibrium.⁴⁵ A typical experiment proceeds by selective inversion of the spin population for a unique resonance, effectively labeling those nuclei via magnetization. This is followed by a variable delay time, during which the chemical-exchange process causes a measurable magnetization transfer of the spin-labeled nuclei between the exchange-related chemical environments, followed by the acquisition of a 1D spectrum. This process is repeated for a series of increasing delay times to generate a profile of magnetization transfer over time. The chemical exchange between the inverted signal and the exchanging signal results in signal attenuation in the exchanging resonance and accelerated relaxation in the inverted signal. By modeling these rates in conjunction with independently measured T_1 relaxation times, the rate of chemical exchange can be extracted.

Although SIR NMR experiments are typically performed on exchange reactions between two chemically distinct environ-

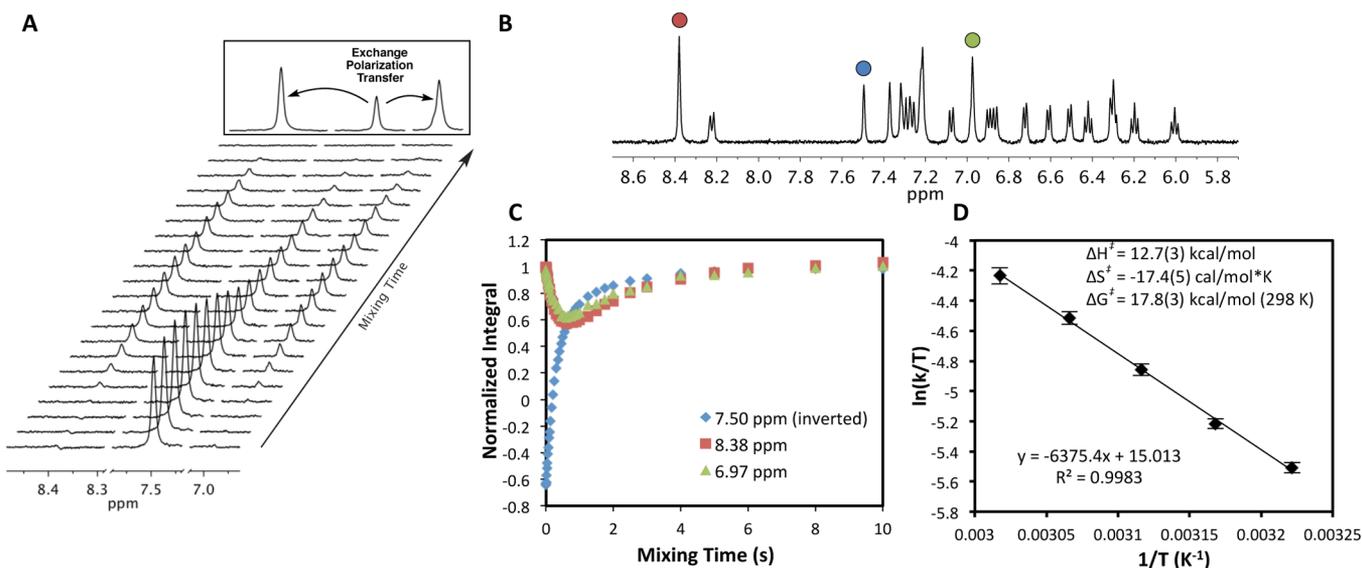


Figure 6. (A) Total magnetization (difference in integration at equilibrium and various mixing times) for the selectively inverted proton resonance (7.50 ppm) and two resonances related by chemical exchange (8.38 and 6.97 ppm) for a representative SIR experiment. (B) ¹H NMR spectrum of 3 indicating the resonance selectively inverted (blue circles) and the resonances attenuated as a result of chemical exchange (red and green circles). (C) Normalized integral as a function of mixing time for the three resonances related by chemical exchange. (D) Eyring plot for the degenerate isomerization of $\Delta\Delta\Delta\Delta$ -3, generated by SIR experiments at various temperatures.

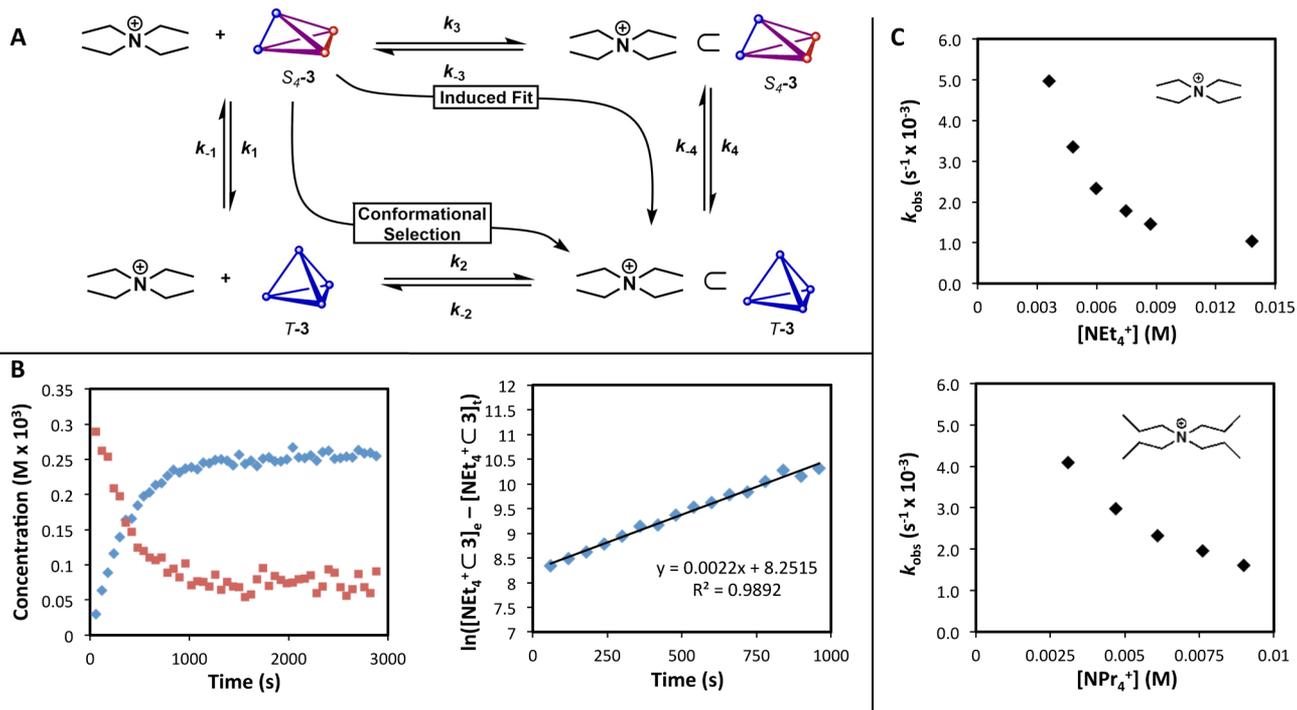


Figure 7. (A) Induced fit and conformational selection mechanism for encapsulation of ammonium guests by cluster 3. (B) (Left) Kinetic profile for approach to equilibrium of tetraethylammonium encapsulation in methanol at 8 °C (blue circles are $\text{NET}_4^+ \text{C} T\text{-}3$, red squares are $\text{NET}_4^+ \text{C} S_4\text{-}3$). (Right) Natural log plot displaying first-order kinetics for approach to equilibrium. (C) Rate dependence of the approach to encapsulation equilibrium on the concentration of tetraethylammonium ion concentration (top) and tetrapropylammonium ion concentration (bottom) for cluster 3.

ments, the data collection and analysis were extended to evaluate the 3-fold symmetric exchange of ligand resonances upon isomerization of host 3. Inversion of the singlet appearing in the ¹H NMR spectrum of 3 at 7.50 ppm resulted in the attenuation of signal intensity for the resonances at 8.38 and 6.97 ppm in a manner characteristic of symmetric 3-fold exchange. The rates of this process were measured at

temperatures from 33 to 53 °C to extract the kinetic parameters of activation by Eyring analysis (Figure 6D). This analysis afforded an enthalpy of activation (ΔH^\ddagger) of 12.7(3) kcal/mol and an entropy of activation (ΔS^\ddagger) of $-17.4(5)$ cal/mol*K, corresponding to a free energy of activation (ΔG^\ddagger) of 17.8(3) kcal/mol at 298 K.^{46,47}

These data show that the host isomerization event is significantly entropically disfavored, indicating an ordered transition state. These observations are consistent with the expected parameters for a consecutive Bailar twist mechanism for the degenerate isomerization of S_4 -symmetric **3** ($\Delta\Delta\Lambda\Lambda$). In comparison, the isomerization of a mononuclear Ga(III) triscatecholate proceeds with an enthalpy of activation (ΔH^\ddagger) of 11.0 kcal/mol, an entropy of activation (ΔS^\ddagger) of -11.4 cal/mol*K, and a free energy of activation (ΔG^\ddagger) of 14.4 kcal/mol at 298 K.⁴³ The activation parameters for these two processes are very similar, and the slight increase of both the entropy and enthalpy of activation for isomerization of **3** is attributed to the effect of minor mechanical coupling by the polycyclic framework of the host.⁴⁸ Having established this species as the S_4 -symmetric M_4L_4 **3** of mixed chirality, the generality of this phenomenon was further interrogated and established by the synthesis of a new assembly bearing similar design features, in addition to a survey of encapsulation behavior for neutral guests (see the [Supporting Information](#)).

Evaluation of the Conformational Selection Mechanism for Guest Binding. To understand the mechanism by which the addition of a guest effects the isomerization of host **3** from the S_4 -symmetric conformation to the T -symmetric isomer, the rate dependence of the approach to the encapsulation equilibrium with respect to guest concentration was studied. A number of supramolecular systems have been shown to undergo configurational changes in order to accommodate and conform to their respective guest molecules.^{49–58} This phenomenon is often referred to imprecisely as “induced fit” binding in reference to the overall shift in host conformation, rather than the mechanism by which this conformational change takes place. To the best of our knowledge, no mechanistic studies have been performed on synthetic hosts to differentiate between an induced fit and conformational selection binding mechanism. By analogy to enzymatic substrate binding, supramolecular assembly **3** might display either induced fit or conformational selection behavior upon binding of ammonium guests ([Figure 7A](#)).

It was possible to observe the approach to encapsulation equilibrium by cooling a methanolic solution of empty host **3** in an NMR tube to -78 °C, followed by addition of the guest at low temperature and then allowing the resulting mixture to warm in a precooled NMR spectrometer. At 8 °C, the approach to equilibrium occurred on an appropriate time scale for kinetics study, and this relaxation was observed to follow pseudo-first order kinetics ([Figure 7B](#)).

The rate of approach to encapsulation equilibrium (k_{obs}) for both tetraethylammonium and tetrapropylammonium guests and host **3** was found to be inhibited by the concentration of guest ([Figure 7C](#)). Guest inhibition of the encapsulation relaxation rate is the classic kinetic signature of a conformational selection mechanism of molecular recognition (vide supra). If the rapid equilibrium approximation—guest exchange is rapid compared to host isomerization—holds true, then the rate constant for approach to equilibrium is shown in [eq 4](#) on the basis of the mechanism depicted in [Figure 7A](#) and by analogy to the established analysis of enzymatic conformational selection. The observation of inhibition with respect to the concentration of ammonium is consistent with [eq 4](#), supporting the conformational selection mechanism of guest binding for assembly **3**.

$$k_{\text{obs}} = k_1 + k_{-1} \frac{1}{1 + \frac{k_2}{k_{-2}} [\text{NR}_4^+]} \quad (4)$$

To evaluate the applicability of the rapid equilibrium approximation for this system, the rate of guest self-exchange for tetraethylammonium with the inclusion complex $\text{NEt}_4^+ \subset \mathbf{3}$ was evaluated by SIR NMR spectroscopy. Eyring analysis of this system revealed that the barrier of guest self-exchange ($\Delta G^\ddagger = 16.2(8)$ kcal/mol at 298 K) is dominated by entropic contributions ($\Delta S^\ddagger = -46.3(30)$ cal/mol*K) with only a modest enthalpic component ($\Delta H^\ddagger = 2.3(1)$ kcal/mol) (see the [Supporting Information](#)). These observations are consistent with the previous analysis of guest self-exchange in host **1**, where a significant entropic contribution to the barrier was observed.⁵⁹ The measured kinetic parameters for guest self-exchange and degenerate host isomerization were extrapolated to 8 °C as an indicator of whether the rapid equilibrium approximation was valid. At this temperature, guest self-exchange occurs with a barrier of 15.3(8) kcal/mol, as compared to 17.6(3) kcal/mol for degenerate host isomerization, implying that guest self-exchange occurs at a rate greater than 60-fold faster than that of host isomerization. As noted, the degenerate host isomerization is only an approximation for the isomerization process for T -symmetric guest binding; however, these processes are mechanistically related, and their activation barriers should be similar. This analysis supports the application of the rapid equilibrium approximation in this system.

However, while the observed guest-inhibited relaxation rates provide strong support for a conformational selection mechanism for guest binding, this does not preclude the possibility of fast, reversible guest association to the low-symmetry host before equilibrium is reached. Indeed, there is some support for the encapsulation of guest by the S_4 -symmetric host, as evidenced by the large change in the ^1H NMR chemical shift of the ammonium ion in the presence of S_4 -symmetric host (see the [Supporting Information](#)). As the low-symmetry host is consumed, a concomitant decrease in the magnitude of the ammonium ion chemical shift is observed. This is consistent with the ammonium encapsulation or external association by S_4 -**3** that is fast and reversible on the NMR measurement time scale.

In light of this evidence, the first guest association step of the induced fit pathway is then kinetically viable, while the second isomerization step is prohibited by the encapsulated guest. Because the isomerization proceeds via stepwise Bailar twists, we hypothesize that the C_3 -symmetric prismatic transition state of the Bailar twist constricts the cavity volume sufficiently that guest encapsulation inhibits the overall process, leading to conformational selection.

Further evidence for guest association to the S_4 -symmetric host is gathered from the interaction between **3** and tetramethylammonium. Tetramethylammonium, presumably due to decreased steric demand compared to its ethyl and propyl-congeners, does not induce an increase in symmetry in **3**. However, shifts in the ^1H NMR spectrum indicate association of the guest to the low-symmetry host. It was hypothesized that if the ethyl- and propylammonium guests were acting as inhibitors to the Bailar twist isomerization mechanism, thus preventing the induced fit mechanism of guest binding, then the addition of tetramethylammonium to S_4 -**3**

would also inhibit the rate at which degenerate isomerization occurred in the low symmetry host.

To evaluate this hypothesis, we again turned to SIR NMR to study the rates of degenerate S_4 -3 isomerization in the presence of the tetramethylammonium guest. Indeed, it was observed that increasing concentrations of ammonium ion had an inhibitory effect on the rate of degenerate self-exchange for S_4 -3, further supporting the hypothesis that a guest molecule disfavors the induced fit pathway by increasing the barrier to the Bailar twist isomerization mechanism (Figure 8).⁶⁰

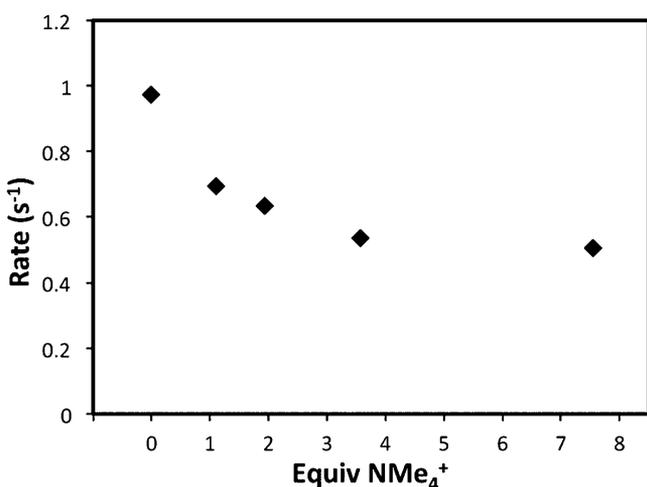


Figure 8. Rate of degenerate self-exchange of low-symmetry host 3 in the presence of various concentrations of tetramethylammonium guest.

Further evidence for reversible preassociation of tetraethylammonium to low symmetry host 3 prior to isomerization can also be inferred from evaluating the guest concentration dependence on the pseudo-zero order initial rate of approach to encapsulation equilibrium, as opposed to the first-order rate constant, which describes the extended kinetic profile. As previously described, the observed rate constant for a reversible reaction is the sum of the forward and backward rate constants. Inspection of eq 4 reveals that the forward rate constant contribution (eq 4, first term) is independent of guest concentration, while the reverse rate constant contributes the guest inhibition to the overall rate constant (eq 4, second term). If the conformational selection mechanism (Figure 7A) is amended to include reversible guest binding for the low symmetry host (k_3/k_{-3}), a new expression for k_{obs} is derived, as shown in eq 5.

$$k_{\text{obs}} = k_1 \frac{1}{1 + \frac{k_3}{k_{-3}}[\text{NR}_4^+]} + k_{-1} \frac{1}{1 + \frac{k_2}{k_{-2}}[\text{NR}_4^+]} \quad (5)$$

In this alternative expression of the observed rate constant, both the forward and reverse reaction display inhibition with respect to the concentration of the guest. The initial rate of the reaction is dominated by contributions from the forward direction (eq 4 and 5, first term) due to the lack of appreciable product accumulation. It is therefore possible to discern these mechanisms by assessing the effect of guest concentration in the initial rate of the approach to encapsulation equilibrium. By isolating the contribution from the forward reaction, an absence of inhibition in the initial rates regime would indicate a simple conformational selection mechanism, while guest-dependent

inhibition of initial rates would provide evidence for fast and reversible association to S_4 -3 in addition to the conformational selection pathway.

Repetition of the encapsulation kinetics experiments at decreased temperature allowed accurate determination of the pseudo-zero-order initial rate as a function of ammonium concentration, which displayed clear inhibition by the ammonium guest (Figure 9). This observation underscores

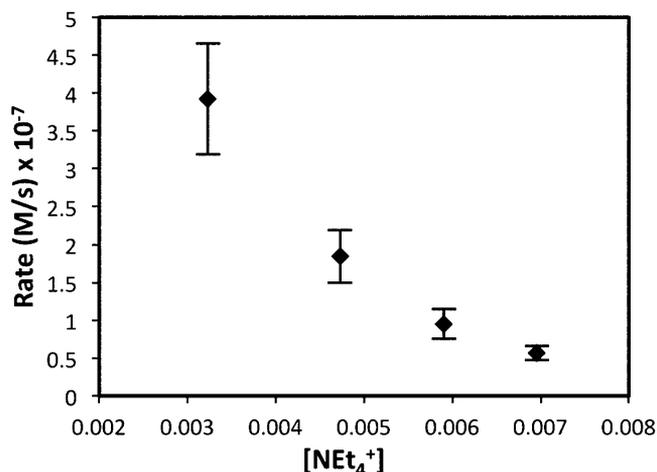


Figure 9. Initial rates for approach to encapsulation equilibrium as a function of the concentration of tetraethylammonium ion concentration for host 3.⁶¹

the conclusion that a complete mechanistic picture for the relaxation to guest binding equilibrium for host 3 involves guest dissociation from S_4 -symmetric $\Delta\Delta\Lambda\Lambda$ -3, followed by host isomerization, and guest binding to T -symmetric homochiral 3. Overall, this constitutes the first observation of a conformational selection mechanism in a synthetic host. Furthermore, identification of the mechanism for host rearrangement as stepwise Bailar twists provides a rationale for why conformational selection is preferred over induced fit binding.

CONCLUSIONS

Understanding the mechanism of binding for molecular recognition processes is essential for the rational design of novel host-guest systems, whether in the biological context of drug design or in the context of synthetic supramolecular sensors and catalysts. In this work, a previously unknown motif in supramolecular metal-ligand self-assembly was identified which gave rise to configurationally dynamic behavior upon recognition of a tetraalkylammonium guest. This feature was taken advantage of in order to study the mechanism by which encapsulation of guests takes place. Specifically, the introduction of appropriate flexibility into the ligand structure of a self-assembled tetrahedral M_4L_4 supramolecular cluster leads to a thermodynamic preference for the $\Delta\Delta\Lambda\Lambda$ -mixed metal center chirality state of S_4 -symmetry. The addition of guest molecules of sufficient steric bulk induces a structural rearrangement to afford the homochiral host of overall T symmetry. It was found that the rate at which this increase in symmetry occurs is inversely correlated to the concentration of the guest molecule, which is singularly consistent with a conformational selection mechanism of molecular recognition. This behavior is known to be the operant mechanism of substrate binding for many biological systems and reinforces the intimate relationship

between synthetic and biological supramolecular systems. In particular, this work highlights the useful role of synthetic supramolecular analogs to biological systems and their application as model systems for biology.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b03812.

Experimental details, synthetic procedures, kinetic rate constant derivations, NMR and HRMS data, and other data (PDF)

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Notes

The authors declare no competing financial interest.

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- (14) For a simple reversible reaction, the first-order rate constant that describes approach to equilibrium is equal to the sum of the forward and reverse rate constants. Reversible bimolecular reactions simplify to this form when the concentration of one of the components is sufficiently large, which applies in this case due to the presumed excess of substrate relative to enzyme.
- (15) It is important to note that not all binding events that proceed via conformational selection show ligand inhibition; when the rapid equilibrium approximation does not hold, approximately first order or saturation behavior can be observed. However, if ligand inhibition is observed, the induced fit mechanism can be definitively ruled out in favor of conformational selection. Finally, it is important to note that many biological systems follow a much more complicated mechanism that may incorporate elements of both conformational selection and induced fit.
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(46) Estimated standard deviations are reported in least significant digits.

(47) The limited temperature range in which these rates could be measured accurately should be taken into account when considering the activation parameters extracted from this Eyring analysis.

(48) The data presented here do not explicitly rule out the “collapsed” D_2 -symmetric host structure; however it is more consistent with the expected observations for a double Bailar twist. In conjunction with literature precedents for self-assembled tetrahedra of mixed chirality, as well as the behavior of the host in the presence of tetramethylammonium (vide infra), this hypothesis is favored.

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(60) Furthermore, this observation provides further evidence to discount the possibility that the low-symmetry host represents a homochiral M_4L_4 structure that has collapsed into D_2 symmetry, since degenerate self-exchange from this structure would be expected to increase in rate in the presence of an internal guest molecule.

(61) Due to the higher error in initial rates measurements, these data were collected in triplicate.